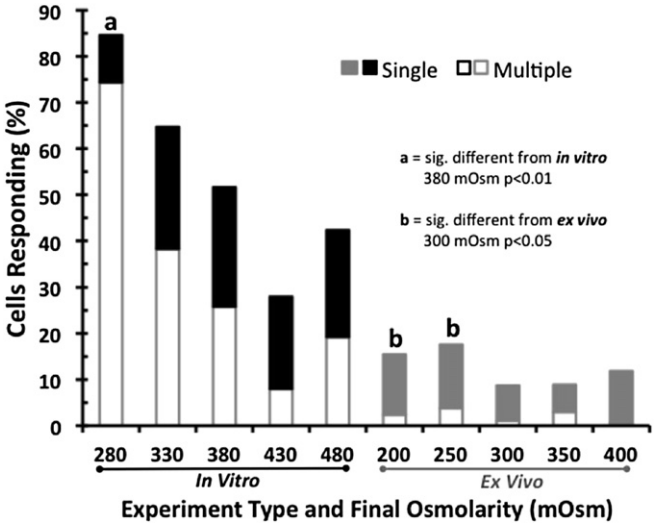

Figure 1. The percentage of chondrocytes responding with single or multiple $[Ca^{2+}]^i$ fluxes *in vitro and ex vivo* as a function of final osmolarity. Starting osmolarity: 380 mOsm (*in vitro*), 300 mOsm (*ex vivo*). n>26 for each osmolarity.

Conclusions: More chondrocytes respond with $[Ca^{2+}]^i$ flux *in vitro* compared to *ex vivo* and those responses are primarily multiple $[Ca^{2+}]^i$ fluxes *in vitro* in contrast to single $[Ca^{2+}]^i$ fluxes *ex vivo*. These findings may indicate a role for the extracellular matrix in i) shielding chondrocytes from the fluid flow occurring during media infusion/withdrawal and/or ii) disabling the chondrocyte ability to harness $[Ca^{2+}]^i$ stores that are likely responsible for multiple/oscillating $[Ca^{2+}]^i$ flux, perhaps via integrin attachment to the matrix. Interestingly, the effect of hypo-osmotic stress on $[Ca^{2+}]^i$ flux was present both *in vitro* and *ex vivo* suggesting that this response, likely mediated by transient receptor potential vanilloid 4, is independent of matrix attachment. In contrast, hyper-osmotic stress failed to elicit a $[Ca^{2+}]^i$ flux *in vitro* or *ex vivo*. Past studies have reported $[Ca^{2+}]^i$ flux responses from chondrocytes *in vitro* exposed to >140 mOsm hyper-osmotic challenge, perhaps suggesting a minimum threshold needed for hyper-osmotic response to occur. In conclusion, we have shown that chondrocyte $[Ca^{2+}]^i$ flux response to hypo-osmotic stress is independent of matrix attachment, however the matrix likely shields the chondrocytes from fluid flow, disabling the release of Ca^{2+} from intracellular stores.

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FN-F INDUCED CATABOLIC ACTIVITIES ARE DEPENDENT ON OXYGEN TENSION IN CHONDROCYTE/AGAROSE CONSTRUCTS SUBJECTED TO BIOMECHANICAL SIGNALS

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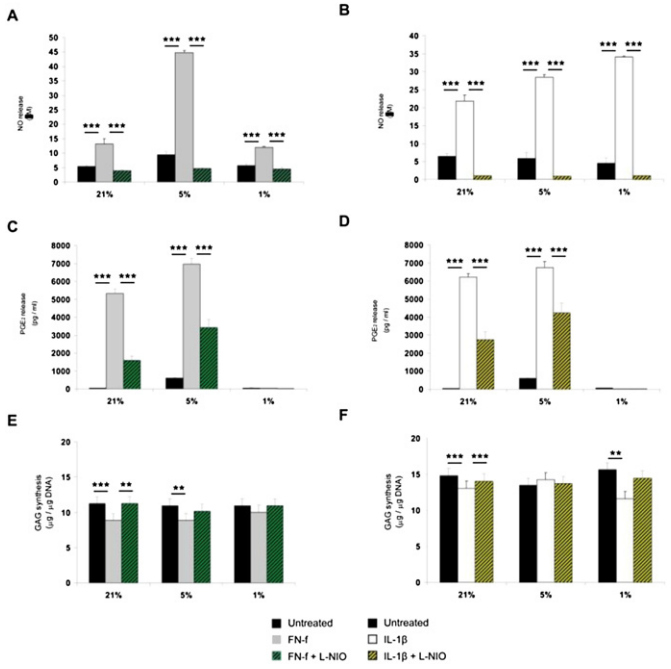
Purpose: There is increasing evidence that the fragments of fibronectin mediate the breakdown of articular cartilage and are potentially the cause of the early damaging effects in osteoarthritis (OA). In particular, the competing mechanisms for the catabolic and anabolic factors, driven respectively by fibronectin fragments (FN-fs) and mechanical loading will influence the balance of these pathways. The present study examined the effects of FN-fs on catabolic activities in chondrocyte/agarose constructs subjected to dynamic compression under different oxygen tension.
Methods: Chondrocyte/agarose constructs were cultured under free-swelling conditions or subjected to dynamic compression (15 %, 1 Hz) with 0 or 1 μM amino-terminal Fn-f (29 kDa) and / or 1 mM L-NIO (inhibits NOS) at 1, 5 and 21 % oxygen tension (v/v) for up to 48 hr. In addition, constructs were cultured with 0 or 10 ng / ml IL-1β and / or L-NIO to compare the effects of the cytokine with FN-fs. NO production, PGE_2 release and GAG synthesis were quantified using biochemical assays. MMP activity was analysed using a fluorogenic substrate assay. Real-time qPCR assays coupled with molecular beacons were used to quantify gene expression of catabolic (iNOS, COX-2) and anabolic (aggrecan, collagen type II) signals by normalizing each target to GAPDH. 2-way ANOVA and a *post hoc* Bonferroni-corrected *t*-test were used to analyse the data.
Results: Both Fn-fs and IL-1β significantly increased the levels of NO, PGE_2 and MMP activity ($p<0.001$) in constructs cultured under 21 % oxygen (Fig. 1). The catabolic response was significantly enhanced in FN-f treated constructs cultured under 5 % oxygen tension and the response was reduced in the presence of the NOS inhibitor (all $p<0.001$). In addition, the presence of FN-fs but not IL-1β significantly inhibits GAG synthesis at 5 ($p<0.01$) or 21 % oxygen tension ($p<0.001$) in constructs cultured for 48 hr. In unstrained constructs, FN-fs or IL-1β increased the levels of NO, PGE_2 and MMP activity and expression of iNOS and COX-2 an oxygen dependent manner with maximal levels at 5 % (all $p<0.001$). The application of dynamic compression reduced catabolic activities and the response was further reduced with L-NIO. Dynamic compression increased GAG synthesis ($p<0.001$) and gene expression of aggrecan and collagen type II and the response was inhibited with FN-fs or IL-1β at 5 or 21 % oxygen tension. The catabolic effects were restored with the application of dynamic compression.
Conclusions: The present findings demonstrate that FN-fs stimulate catabolic activities via an iNOS dependent pathway, resulting in NO production, MMP activity, and PGE_2 release. The effect of FN-fs was more potent than the cytokine and the response was dependent on oxygen tension. In addition, stimulation with biomechanical signals reduced catabolic activities and co-stimulation with the NOS inhibitor abolished FN-f induced catabolic response. Interestingly, low oxygen tension (5 %) exacerbated FN-f induced catabolic activities, but did not affect the loading-induced recovery. These findings indicate that FN-fs exert catabolic effects in an oxygen dependent manner and the response was prevented with biomechanical signals. The combination of mechanical and pharmacological interventions with NOS inhibitors makes this study a useful tool to examine further the interactions of biomechanics and cell signalling in OA.



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EFFECT OF ZOLEDRONIC ACID (ZA) ON CALCIUM SIGNALING OF CHONDROCYTES UNDER FLUID FLOW

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Purpose: Zoledronic Acid (ZA) is a bisphosphonate drug approved by FDA which is widely used to treat osteoclast resorption related bone disease. In previous studies, we found that ZA could significantly suppress the development of post-traumatic osteoarthritis (PTOA). After destabilization of the medial meniscus (DMM), mice with ZA injections showed much less signs of OA than the control group (Fig. 1). However, the chondro-protective mechanisms of ZA in PTOA are not